HUNTINGDON LIFE SCIENCES LTD
PO BOX 2
HUNTINGDON
CAMBRIDGESHIRE
PE18 6ES
ENGLAND

Report No.:

Title:

Study No.:

External Testing Facility No.:

Test Substance:

Study Director:

Sponsor:

Sponsor Representative:

Testing Facility: Huntingdon Life Sciences Ltd
PO Box 2
Huntingdon
Cambridgeshire
PE18 6ES
ENGLAND

Study Completion Date: February 25, 2000

Security Statement:

TABLE OF CONTENTS

ABSTRACT

The study was designed to assess the toxicity of the test substance following a single dermal dose to the rabbit.

The procedure used is described in this report. The procedure complies with that described in the United States Environmental Protection Agency OPPTS Testing Guidelines for Health Effects (Series 870) which came into force during August 1998.

was administered as supplied at a dose level of 2000 mg/kg bodyweight to the dorsol-lumbar region of five male and five female rabbits. The treated area of skin was washed with a warm mild detergent solution in water 24 hours after application of the test substance. Rabbits were assessed for dermal irritation from Day 2 (following test substance administration/removal of the dressings) through to Day 15 (study termination).

There were no deaths and with the exception of faecal disturbance (few faeces) in one female rabbit on Day 3, there was no evidence of any systemic response to treatment in any animal during the study.

Persistent slight to moderate irritation (erythema with or without oedema up to Grade 3) was evident in all rabbits following removal of the dressings and over the following days. These reactions had notably ameliorated by the second week of the study with resolution in all but three animals complete by Day 15. In the three remaining rabbits slight erythema (Grade 1) was still evident at study termination. Also notable in all rabbits during the first days following treatment was a very dry texture to the skin over the treatment site, desquamation of the skin on the treatment site (notable in all rabbits and present in six rabbits at study termination) and in one rabbit localised necrosis/blanching evident throughout the observation period.

Bodyweight gains in the majority of animals were considered satisfactory throughout the study. A slight weight loss was recorded for two females on Day 8, with a more notable weight loss in a further female on Day 15 these findings were not considered to be of any toxicological significance.

Macroscopic examination revealed no abnormalities.

Under the conditions of this study, the acute lethal dermal dose to rabbits of was demonstrated to be greater than 2000 mg/kg bodyweight.

GLP COMPLIANCE STATEMENT

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

The UK Good Laboratory Practice Regulations 1997 (Statutory Instrument No 654) and from 14 December 1999, the UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No 3106).

EC Council Directive, 87/18/EEC of 18 December 1986, (No. L 15/29).

The raw data has been reviewed by the Study Director, who certifies that the information contained in this report is consistent with and supported by the raw data.

25 February 2000

Date

Study Director,
Huntingdon Life Sciences Ltd.

QUALITY ASSURANCE STATEMENT

Study Title: Acute Dermal Toxicity of in the Rabbit

Study Number:

Study Director:

This study has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Inspections were made by the Quality Assurance Department of various phases of the study conducted at Huntingdon and described in this report. The dates on which the inspections were made and the dates on which the findings were reported to the Study Director and to Management, Huntingdon Life Sciences are given below.

Study Phases Inspected	**Date of Inspection**	**Date of Reporting**
Protocol Review	14 August 1998	14 August 1998
Treatment procedure	25 September 1998	25 September 1998
Scoring	25 September 1998	25 September 1998
Records audit	25 September 1998	25 September 1998
Report	05 February 1999	11 February 1999

14 February 2000
Date

Quality Assurance Group Manager,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

APPROVAL SIGNATURES

This report consist of Pages 1 through 14 including Tables 1-3 and Appendix 1.

Management,
Huntingdon Life Sciences Ltd.

25 Feb 00
Date

Study Director,
Department of Acute Toxicology,
Huntingdon Life Sciences Ltd.

25 February 2000
Date

Sponsor Representative.

21 Jan. 2000
Date

STUDY INFORMATION

Study Initiation Date	September 14, 1998
Experimental Start Date:	September 24, 1998
Experimental Termination Date:	October 08, 1998
Study Completion Date:	February 25, 2000
Study Director:	
Sponsor Representative:	
Sponsor:	
Senior Technician for study:	
Head, Department of Acute Toxicology:	
Chief Technician - Acute Toxicology:	
Director Quality Practices:	
Head, Department of Analytical Chemistry & Pharmacy:	
Head, Department of Microbiology	
Principal Veterinarian & Animal Care & Welfare Director:	

Acute Dermal Toxicity of in the Rabbit

I. INTRODUCTION

The study was designed to assess the toxicity of following a single dermal dose to the rabbit.

The duration of the study was fourteen days, in that animals were dosed on Day 1, observed subsequently for 14 days with study termination on Day 15.

II. MATERIALS AND METHODS

A. Test Substance: lot number BN028339, was received at Huntingdon Life Sciences on April 06 1998. The test substance a pale yellow liquid was stored at room temperature (ambient temperature between 10 and 30°C). The Huntingdon Test Substance Data Sheet indicated that the test substance was stable until February 28, 2001. The test substance, as received, is regarded as the "pure" material and representative of All the remaining test substance will be returned to the sponsor after the completion of all the relevant studies, with the exception of a 1 g sample which will be retained by Huntingdon Life Sciences. The absorption of the test substance was not quantitated. Test substance characterisation has been carried out by the Sponsor Study number).

B. Dosage Formulation: The test substance was administered undiluted (specific gravity 1.0094). Individual 1.982 ml/kg bodyweight doses were placed directly onto the dorsol-lumbar region of each rabbit using 1 and 5 ml plastic syringes and a glass rod for spreading the test substance on a 100 mm x 100 mm dose site.

C. Animals: Equal numbers of male and female New Zealand White rabbits, in the weight range 2093 g to 2741 g, were purchased from Harlan UK Ltd, Bicester, Oxon, England for use in this study. The rabbits were received on September 10, 1998. All the rabbits were kept in isolation throughout the study. Within the first 48 hours following arrival, for environmental enrichment each animal was give a small amount of autoclaved hay. The rabbits were acclimatised for 14 days during which time they were observed daily for signs of ill-health (this data was reviewed by a veterinary officer before the animals were selected for study dosing). Each animal on arrival was identified by a numbered aluminium tag placed through the edge of one ear. This number was unique within the Huntingdon Life Sciences Acute Toxicology Department throughout the duration of the study. Each cage was identified by a coloured label displaying the study schedule number, animal number and initials of the Study Director and Home Office licensee. This particular test system and the specific route by which the test substance was administered were chosen for the following reasons: 1) the rabbit was chosen as the test species as it has been shown to be a suitable model for this type of study and is one of the species recommended in the test guideline and 2) topical application corresponds to a potential route by which humans may be exposed to the test substance. The number of animals used for this study was based upon testing guideline requirements.

D. Food and Water: The rabbit was provided, *ad libitum*, with a standard laboratory diet, SDS Stanrab (P) SQC Rabbit Diet (supplier: Special Diet Services Ltd, Witham, Essex) and drinking water via an automatic watering system (supplier: Anglian Water). The batches of diet were analysed once, by the supplier, for nutrients, possible contaminants and micro-organisms, likely to be present in the diet, and which, if in excess may have an undesirable effect on the test system. Results of routine physical and chemical analyses of drinking water performed by the supplier were made available to Huntingdon Life Sciences Ltd. as quarterly summaries. Water was supplied in conformity with EC Directive 80/778/EEC and UK Water Act 1989 and subsequent amendments. No contaminants capable of adversely affecting the integrity or interpretation of the results from this study were known to be present in the basal diet or the drinking water during the conduct of this study. The Study Director reviewed the feed and water analyses. The certificate of analyses will be lodged in Huntingdon Life Sciences Ltd. Archives. Samples of water were taken from the drinking water at source in the animal room prior to the study start. The samples were analysed for microbial contaminants (total viable count, coliform count and *E.Coli* count) by Huntingdon Life Sciences Ltd., Department of Microbiology. A certificate of analysis is appended to this report.

E. Housing and Environment: The rabbits were housed individually in suspended metal cages with perforated floors measuring 45.5 cm high, 76 cm wide and 60.5 cm deep (floor area 4598 cm^2). Absorbent cage liners were placed in the pan below the metal mesh floor of the animal cage to absorb liquids. During the treatment phase of the study, the air-conditioned animal room temperature and relative humidity were recorded using a seven day recorder. These parameters ranged from 19 to 22°C and 40 to 65%, respectively. Air exchange is set to provide approximately 15-19 air changes per hour and fluorescent lighting was controlled by a time switch to provide 12 hours of artificial light (0700-1900 hours) in each 24-hour period.

F. Methods:

1. Animals: Five males and five females (all nulliparous and non-pregnant) were randomly allocated by bodyweight, so that the group mean bodyweights were approximately equalised, using a computer programme. Animals were in the bodyweight range 2494 g to 2828 g and at least 12 weeks of age prior to dosing on Day 1 of the study.

2. Preparation: The day prior to treatment hair was removed with electric clippers from the dorso-lumbar region of each rabbit exposing an area of skin approximately 10% of the total body surface area. Only animals with healthy intact skin were used. The skin was not abraded.

3. Dosing: On the day of treatment (September 24, 1998), the test substance formulation was applied by spreading it evenly over the prepared skin using a glass rod. The treatment area (approximately 100 mm x 100 mm) was covered with porous (<8 ply) gauze held in place with a non irritating dressing, and further covered by a waterproof dressing encircled firmly around the trunk of the animal. At the end of the 24 hours exposure period the dressings were carefully removed and the treated area of skin was washed with a warm mild detergent solution (Brio, Morris & Co (Shrewsbury) Ltd, England) in water (34°C) to remove any residual test substance. The treated area was blotted dry with absorbent paper. To prevent any possible ingestion of dose residue for the remainder of the working day of Day 2 animals were fitted with plastic Elizabethan collars.

4. Observations: All rabbits were observed twice daily for mortality and morbidity.

5. Body Weights: The bodyweight of each rabbit was recorded on Days 1 (prior to dosing), 8 and 15. Individual weekly bodyweight changes and group mean bodyweights were calculated.

6. Clinical signs: Animals were observed immediately after dosing and at approximately hourly intervals for the remainder of Day 1. On subsequent days animals were observed once in the morning and again at the end of the experimental day (with the exception of Day 15 - morning only). The nature and severity of the clinical signs and time were recorded at each observation. All animals were observed for 14 days after dosing.

7. Dermal Responses: The treated skin of each rabbit was examined once daily on Day 2 through to Day 15. At each interval, dermal irritation was assessed for erythema, eschar formation, oedema and any other lesion. Animals were clipped as needed to evaluate dermal responses.

Dermal responses at the treatment site were assessed using the following arbitrary numerical scoring system (based on Draize J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, Assoc. Food & Drug Officials of the US, Austin, TX, 1959.

Erythema and eschar formation:	
No erythema	0
Slight erythema	1
Well defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema formation:	
No oedema	0
Slight oedema	1
Well-defined oedema (area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

Any lesion not covered by this scoring system was described

8. Animal Disposition: After the final observation (October 08, 1998) all the rabbits were sacrificed by an intravenous overdose of phenobarbitone sodium B.P. 200 mg/ml (Brand name EUTHATAL manufactured by Rhone Merieux of Harlow, Essex, England). All animals were subjected to a macroscopic examination which consisted of examination of the treated skin site and underlying tissue and opening the cranial, thoracic and abdominal cavities. The macroscopic appearance of all examined organs was recorded.

G. Location of Study records: The protocol and all amendments/deviations as well as all raw data, specimens, sample of the test substance and study related documents generated during the course of the study at Huntingdon Life Sciences Ltd, together with a copy of the final report will be lodged in the Huntingdon Life Sciences Ltd, Archive, Huntingdon, England. Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the sponsor will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the Sponsor's prior approval.

III. RESULTS

A. Mortality: No deaths occurred during the study. The macroscopic examination conducted at study termination revealed no abnormalities (with the exception of persistent dermal responses (as noted in section C) representative skin sections were taken and preserved).

B. Clinical Signs: There were no deaths and with the exception of faecal disturbance (few faeces) in one female rabbit on Day 3, there was no evidence of any systemic response to treatment in any animal during the study.

C. Dermal Responses: Summation of dermal responses is presented in Table 1. Persistent slight to moderate irritation (erythema with or without oedema up to Grade 3) was evident in all rabbits following removal of the dressings and over the following days; these reactions had notably ameliorated by the second week of the study with resolution in all but three animals complete by Day 15. In the three remaining rabbits slight erythema (Grade 1) was still evident at study termination. Also notable in all rabbits during the first days following treatment was a very dry texture to the skin over the treatment site, desquamation of the skin on the treatment site (notable in all rabbits and present in six rabbits at study termination) and in one rabbit localised necrosis/blanching evident throughout the observation period.

D. Body Weights: The weight range for rabbits used in this study was 2494 g to 2828 g at treatment initiation. Bodyweight gains in the majority of animals were considered satisfactory throughout the study. A slight weight loss was recorded for two females on Day 8, with a more notable weight loss in a further female on Day 15 these findings were not considered to be of any toxicological significance. Individual and group mean bodyweights are summarised in Tables 2 and 3.

E. Statistical analysis Group mean bodyweights were calculated using appropriate means (Documenta Geigy, Scientific Tables (1962) 6th Edn, Geigy UK Ltd.). No other statistical analyses were carried out.

F. Deviations: There were no deviations from protocol.

IV. CONCLUSION

Under the conditions of this study, the acute lethal dermal dose to rabbits of was demonstrated to be greater than 2000 mg/kg bodyweight.

V. AMENDMENTS TO PROTOCOL

A protocol amendment was raised to change for regulatory compliance the test guideline referenced in the study protocol, amendment of pre dose bodyweight range, clarification of test substance administration and revision of study details.

VI. TABLE 1

Dermal Reactions

Dosage 2000 mg/kg bodyweight

Rabbit N No. & Sex		Day of study													
		2	3	4	5	6	7	8	9	10	11	12	13	14	15
1267 M	E	2	1	1	1	1	1	1	0	0	0	0	0	0	0
	O	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	#	A	A	A	A	-	-	-	C	C	C	C	C	C	C
1269 M	E	2	2	2	2	2	2	1	1	1	1	1	1	1	1
	O	2	2	2	2	2	2	2	1	1	1	1	0	0	0
	#	A	A	A	A	-	-	-	C	C	C	C	C	C	C
1275 M	E	2	2	2	2	1	1	1	1	1	0	0	0	0	0
	O	2	2	2	1	1	1	1	0	0	0	0	0	0	0
	#	A	A	A	A	-	-	-	C	C	C	C	C	C	C
1276 M	E	2	2	2	2	2	2	2	0	0	0	0	0	0	0
	O	2	2	2	2	2	2	1	0	0	0	0	0	0	0
	#	A	A	A	A	-	-	-	C	C	C	-	-	-	-
1278 M	E	2	2	2	2	2	2	1	1	1	1	1	1	1	1
	O	1	1	1	1	1	1	1	1	1	1	1	1	0	0
	#	AB	AB	AB	ABC	BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
1283 F	E	2	2	2	2	2	1	1	1	1	1	1	1	1	1
	O	2	2	2	2	1	1	0	0	0	0	0	0	0	0
	#	A	A	A	A	-	-	-	-	-	C	C	C	-	-
1286 F	E	3	2	2	1	1	1	1	1	1	1	1	1	0	0
	O	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	#	A	A	A	A	-	-	-	C	C	C	C	C	C	C
1294 F	E	2	2	2	2	2	1	1	1	1	0	0	0	0	0
	O	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	#	A	A	A	A	C	C	C	C	C	C	C	C	-	-
1295 F	E	2	2	2	2	2	1	1	1	1	1	1	1	0	0
	O	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	#	A	A	A	A	C	C	C	C	C	C	C	C	C	C
1299 F	E	2	2	2	3	3	2	1	1	1	0	0	0	0	0
	O	3	2	2	2	2	1	0	0	0	0	0	0	0	0
	#	A	A	A	A	C	C	C	C	C	C	C	C	-	-

Key

E: Erythema O: Oedema #: Other reactions

A: Skin on treatment site very dry (almost dehydrated)

B: Localised necrosis and blanching

C: Dryness, sloughing and /or scaling on treatment site (reported as desquamation)

-: No abnormalities observed

VI. TABLE 2

Individual and group mean bodyweights (g)

Dose (mg/kg)	Sex	Animal Number	Bodyweight (g) at Day		
			1*	8	15
2000	Male	1267	2669	2771	2834
		1269	2675	2784	2993
		1275	2659	2693	2860
		1276	2494	2533	2584
		1278	2702	2816	2923
		Mean	2640	2719	2839
2000	Female	1283	2757	3248	2947
		1286	2828	2819	2891
		1294	2500	2489	2580
		1295	2717	2758	2834
		1299	2706	2796	2941
		Mean	2702	2822	2839

*: Prior to dosing

VI. TABLE 3

Individual bodyweight changes (g)

Dose (mg/kg)	Sex	Animal Number	Bodyweight changes at Day (g)	
			8	15
2000	Male	1267	102	63
		1269	109	209
		1275	34	167
		1276	39	51
		1278	114	107
		Mean	80	119
2000	Female	1283	491	-301
		1286	-9	72
		1294	-11	91
		1295	41	76
		1299	90	145
		Mean	120	17

VII. APPENDIX 1

Certificate of analysis for microbial analysis of drinking water

Huntingdon Life Sciences study number :		
Report number :		
Source of water sample (s):	Huntingdon Research Centre, Building R14, Room 1. (1) Cold water tap, Entry. (2) Cold water tap, Exit.	
Date sampled and tested :	24 March 1998	
Test procedure :		
Research Laboratory	Huntingdon Research Centre Department of Cellular Toxicology P O Box 2 Huntingdon Cambridgeshire PE18 6ES ENGLAND	
RESULTS	**Count**	**Specification**
Total viable count for aerobic bacteria :	(1) <1 cfu/ml (22°C) (2) <1cfu/ml (22°C)	$<10^4$ cfu/ml (22°C)
	(1) 1 cfu/ml (37°C) (2) 1 cfu/ml (37°C)	$<10^2$ cfu/ml (37°C)
Total viable count for coliform bacteria :	(1) <1 cfu/100ml (2) <1 cfu/100ml	<1 cfu/100ml
Total viable count for *E.coli* :	(1) <1 cfu/100ml (2) <1 cfu/100ml	<1 cfu/100ml
CONCLUSION	Samples (1) and (2) showed satisfactory microbiological quality.	
Analyses performed by : **Senior Technician**	**Signature :** **Date :** 2 April 1998	
Results reviewed by : **Head, Microbiology**	**Signature :** **Date :** 2 April 1998	

cfu - colony forming unit